# HOT METAL SWITCH: Synthetic *in vitro* circuit for the detection of heavy metal ions

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## Background

### Lead

Lead is a toxic heavy metal. Lead poisoning causes problems with the brain, liver, and kidneys in adults. Children are especially vulnerable to lead. Lead poisoning in children interferes with development, behavior, and learning. Old pipes contain lead. If the water running through the pipes is not properly treated, it corrodes the pipes and lead enters the water. The recent crisis in Flint, Michigan highlights the need for an inexpensive, easy-to-use sensor that residents can use to test their water whenever they wish.

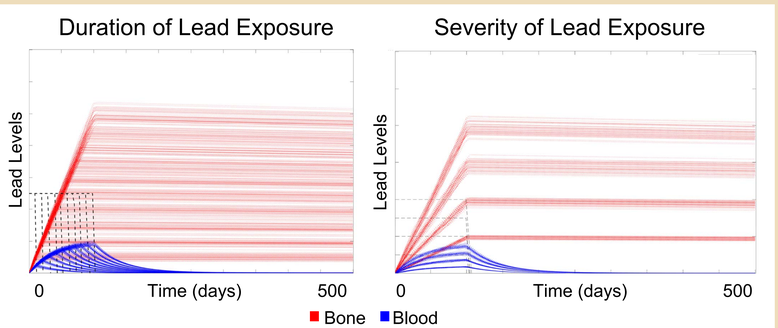
### Paper-based Sensors

The Collins lab at MIT developed paper-based sensors by freeze-drying *in vitro* protein synthesis reactions onto paper. These reactions are made by combining cell-free extract, which contains all the necessary transcriptional and translational elements to make protein from DNA, and template DNA to express. When the freeze-dried reactions were rehydrated, the reactions proceeded normally. The Collins lab added RNA sensors to the cell-free reaction to detect mRNA molecules. In the presence of the target molecules, the paper produced a signal within two hours. We adapted this system to detect heavy metals interfacing it with a lead-sensitive DNAzyme.

## Human Practices

### Modeling: The Need for Early Detection

Correlations between the severity or duration of lead exposure and blood-lead level in children are crucial for situation assessment once elevated lead levels have been detected in water. Using literature values, the amount of lead in the blood and bone was modeled under different conditions.




Regardless of the duration or severity of exposure, lead quickly accumulates in children's bones. Although blood-lead levels decrease quickly, the lead in the bone slowly enters the bloodstream, so the blood-lead level is never zero. Thus, early detection of lead contamination is essential, especially for the health of children.

### Outreach: Raising Awareness

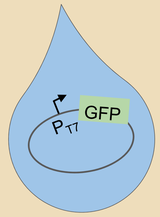
The local public schools recently tested all their water fountains for lead, so we created a short video for the classroom designed to teach kids about the dangers of lead. The video highlighted sources of lead poisoning and ways to prevent lead exposure.

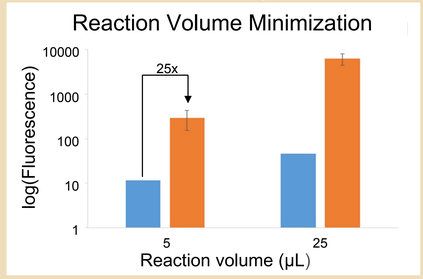
We also visited the Carnegie Science Center and several summer camps to teach kids about DNA and synthetic biology as an approach to solve current global problems, such as metal contamination.


## Sensor Design and Optimization

### Cell-free *in vitro* Protein Synthesis

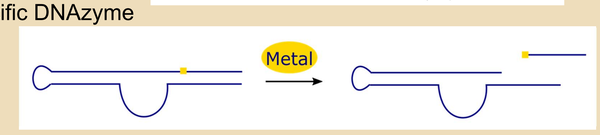
Cell-free T7 extract provides a platform to rapidly test many DNA circuit designs and for the construction of paper-based sensors. It also allows us to use circuit components like DNAzymes and to measure things like heavy metals without worrying about getting them into cells or toxicity. We first minimized the extract reaction volume to save money on materials.



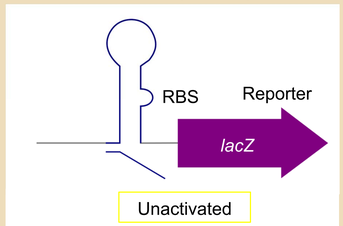



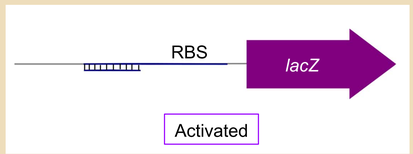

### Circuit

Metal-specific DNAzyme

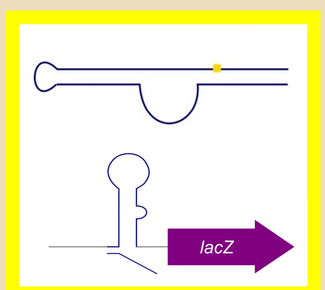


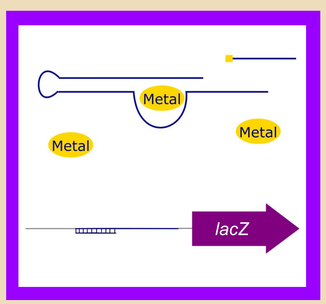

Toehold switch regulating reporter expression (Part BBa_K2084002)



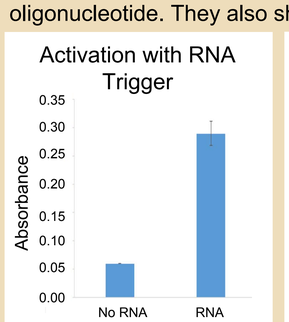



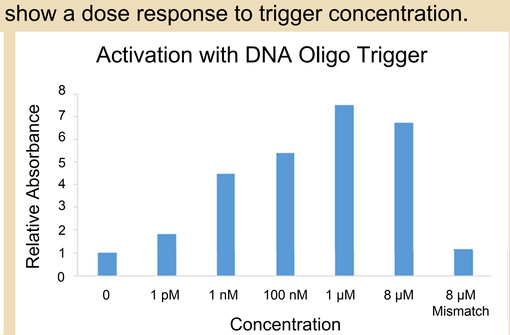

In the presence of the target metal, the DNAzyme cleaves its substrate. The cleavage product activates the toehold switch to express the reporter.



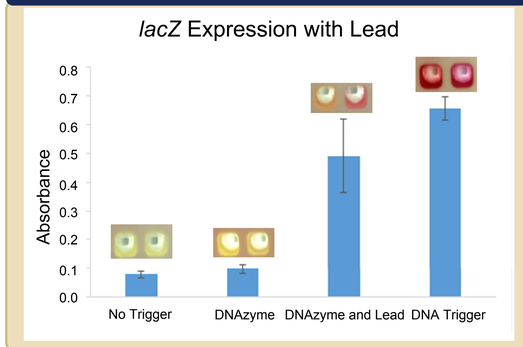




We are the first to show that toehold switches can be activated with a DNA oligonucleotide. They also show a dose response to trigger concentration.




## Lead Sensor Results

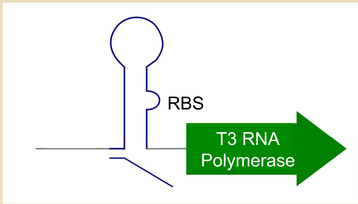


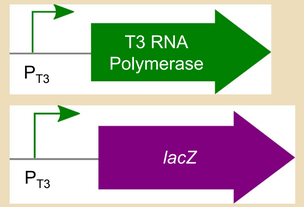

In reactions containing 3.74 nM DNAzyme and 2 µM (~414 ppb) lead, a colorimetric signal was produced. 2 µM is a higher concentration than the EPA MCL of 15 ppb, but it is far lower than the 13,200 ppb found in Flint, Michigan.

## Future Work

### Amplification

An amplification system would increase the expression of the reporter gene and decrease the detection limit of the sensor. The proposed amplification system shown below is triggered by the production of T3 RNA polymerase from the toehold switch. The amplification is self-propagating, and the integration of the system is facilitated by the *in vitro* system.




### Moving the Sensor to Paper

One major advantage of the *in vitro* sensor system is its potential to be paper-based. The reactions may need further optimized to reliably signal the presence of metal as a paper-based sensor.

### Expansion to Different Heavy Metals

The *in vitro* system allows additional flexibility in the target metal. The DNAzyme can be changed to detect any metal, such as thallium, mercury, and arsenic, which might be harmful to wildlife and humans.

## Conclusions

**Hot Metal Switch** shows promising results as a simple, at-home sensor for lead in drinking water. The circuitry can be applied to a wide range of metals to make sensors for many metal contaminants that are harmful to both humans and wildlife. The paper-based medium will make the detector useful in the field as well since it does not require additional equipment and is easy to store.

## Acknowledgements

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